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BROMBERG & SUNSTEIN LLP
125 SUMMER STREET
BOSTON, MA 02110-1618

EXAMINER	
TON, THAIAN N	

ART UNIT	PAPER NUMBER
1632	

DATE MAILED: 11/17/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/995,452	BENVENISTY ET AL.	
	Examiner	Art Unit	
	Thaian N. Ton	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 September 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-9,11-21 and 24-56* is/are pending in the application.

4a) Of the above claim(s) *18-35 and 37-47* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9,11-17,36 and 48-56* is/are rejected.

7)☒ Claim(s) *3,11-17 and 49-56* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Applicants' Request for Continued Examination (RCE) under 37 CFR 1.114, filed 9/22/04, has been entered. Applicants' Amendment, filed 9/22/04, has been entered. Claims 10, 22-23 have been cancelled. Claims 1-9, 11-21, 24-56 are pending. Claims 18-35, 37-47 are withdrawn. Claims 57 and 58 were not entered in the Advisory Action, mailed 7/9/04. Claims 1-9, 11-17, 36, 48-56 are under current examination.

The Benvenisty Declaration (37 CFR §1.132) has been considered but is not found to be persuasive.

Claim Objections

Claim 3 is objected to because there is no verb to describe the protein expression in line 2 of the claim. The claim recites, "the protein not expressed....". Appropriate correction is required.

Claim 11 is objected to for being an improper Markush group. The claim recites, "wherein the transfection preparation further comprises one or more transfection reagents selected from the group consisting of cationic polymer agents." See last sentence of the claim. See MPEP §2173.05(h) for proper Markush language. The recitation of "cationic polymer agents" does not constitute a group. Appropriate correction is required.

Claims 12-17 begin with, "A method according to claim ...". This is objected to because these claims refer back to a specific claim, and recitation of the term, "A method" implies that there is more than one method, where the dependent claims are clearly referring to a specific method. Thus, the recitation of, "The method according to claim ..." would obviate this objection.

Claims 57 and 58 are improper because they do not have a proper identifier. They are currently listed as "not entered." These claims were not entered, as indicated in the Advisory Action, mailed 7/9/04. Furthermore, the text of the claims is not provided. Appropriate correction is required.

Claims 49-56 begin with, "A reagent cell population according to claim ...". This is objected to because they refer back to a specific claim, and reciting the term "A reagent cell population" implies that there is more than one cell population, where the dependent claims are clearly referring to a specific cell population. The recitation of, "The reagent cell population ...." would obviate this objection.

Claim 54 is objected to because it does not end in a period. Appropriate correction is required.

Response to Declaration

The Benvenisty Declaration has been considered but is not found to be persuasive. The Declaration seeks to clarify the record concerning the transfection of human ES cells relative to the transfection of mouse and other animal cells.

Firstly, that having the ability to transfect mouse and other animal ES cells does not successfully translate into transfection of human ES cells successfully, and secondly, why the proposed combinations and modificatiosn set forth in the prior Office action are not obvious and finally, why those of skill in the art would not expect such combinations and modifications of mouse and other animal cell transfection protocols would be successful in achieving efficient transfection of human ES cells. See p. 2, #2.

The Declaration states the following:

> Claims 1 and 11 have now been amended, requiring introduction of the DNA in a transfection preparation, which has transfection reagents selected from cationic non-lipid polymer reagents, non-liposomal reagents, and cationic lipid agents and no other, (*Emphasis added*) meaning that electroporation cannot be used to introduce DNA, and adenovirus cannot be present in the transfection preparation.
> See pp. 2-3, #3.

This is not persuasive. The claim language of claims 1 and 11 specifically recites that a transfection preparation "comprising", which is considered open language. Thus, using the term *comprising* is, "inclusive or open-ended and does not exclude additional, unrecited elements or method steps." See MPEP §2111.03. Thus, it is incorrect to state that no other agent or reagent can be present in the transfection preparation; further, this language allows and encompasses the use of adenoviruses in conjunction with the reagents/agents. It is acknowledged that Smith does not anticipate the claims, as currently amended, directed to methods of producing the transfected human ES cells, because they do not specifically teach

transfection preparations from the group consisting of cationic non-lipid polymer reagent, non-liposomal reagent, a cationic lipid agent, as required. However, the combination of Smith and Fasbender render the instant invention obvious because they teach each recited element in the claims. However, it is maintained that Smith teach the claimed cell populations (claims 36, 48-56), because these are considered product-by-process claims.

The Declaration argues that the combination or modification of the cited references in the prior Office action would not yield successful transfection of HES cells because those working in the field knew that electroporation did not introduce DNA into hES cells at rates or levels high enough to do meaningful research with genetically altered hES cells. Further, the Declaration points to Figure 1 for support that transfection with chemical transfection reagents, such as EXGEN 500™, a polycationic non-lipid polymer, successfully introduced DNA into hES cells at levels over an order magnitude better than other chemical reagents or electroporation. See pp. 3-4, #5. The Declaration further states that Figure 1 shows the relative transfection efficiencies between the various methods examined, and that the levels of transfection in hES cells using cationic polymers versus electroporation protocol. See p. 4, #6. The Declaration argues that the prior Office actions' rejections are based upon the fundamental assumption that translating mouse and other animal transfection protocols, most developed for transfection of cell lines other than ES cells, can easily be adapted to achieve successful and

efficient transfection of human ES cells. See p. 4, #7. The Declaration points to Zwaka who states that human ES cells, the best chemical reagents yield stable transfectants at rates about 10^{-5}; mouse ES cells electroporation procedures yield substantially lower rates. See p. 5, #8. The Declaration argues that the yield of transfected hES cells through electroporation were not feasible for performing further manipulations. See p. 5, #9. The Declaration further argues that typical mouse ES cell electroporation protocol resulted in poor yield of transfected cells that it was not practical to use mouse electroporation protocols to introduce DNA into human cells. Thus, the Declaration concludes that using electroporation is not a viable means for introducing DNA into hES cells. P. 6, #10. The Declaration states that as such, Eiges and Zwaka provide evidence that mouse protocols for introducing DNA into human ES cells did not work at the time this invention was submitted. Thus, they contradict Smith's claim, that in absence of any experimental evidence with human ES cells using electroporation or other means, that essentially any means will work for introducing a marker into any animal ES cells. See p. 6, #11.

This is not persuasive. The Declaration addresses levels or rates of introduction of the DNA into hES cells. Whether or not these levels or rates are useful to do meaningful research, or if the yield of the cells were feasible for further manipulation, are not within the scope of the claims. The claims, as written, encompass even one hES cell that has been transfected by the described methods.

The claims do not have any particular limitation with regard to percentage, concentration or number of cells that would be transfected, nor does the claim require any particular transfection efficiency. Thus, Figure 1, and the Declaration provide support that transfection protocols known in the art are able to transfect hES cells.

The Declaration argues that the cited art provides no protocols for DNA transfection into ES cells, other than mice. For example, Smith may state that some of its techniques are applicable to humans, but the examples are limited to mouse. The art of Myers, Fasbender and Pascolo do not provide insight on thre transfection of hES cells (p. 7, #13). Further, the Declaration states that the lack of any previous protocol for DNA transfection into hES cells is significant because it underscores that murine ES cell techniques cannot be assumed to work in hES cells (p. 7, #14), and that substantial differences exist between mouse and human ES cells (p. 7, #15) such as morphology and growth rate between mouse and human ES cells and further, that electroporation did not work for introducing DNA into hES cells as evidenced by the post-filing art of Zwaka provides evidence that transferring of technology from one species to another is not trivial (p. 8, #16, p. 9 #17). The Declaration states that Eiges shows transfection rates, using ExGEN 500™, an order of magnitude better than using electroporation, LipofectAMINE plus™, or FuGENE™. Further, that the Zwaka reference teaches that successful introduction of DNA into hES cells was "too low to be practical." (p. 10, #19-20).

Finally, the Declaration states that even if the combinations proposed in the 103 rejections were made, one simply would not arrive at the subject matter claimed in the instant application because researchers in the field of hES cell research may have to combine or modify teachings in the prior art of stem cell research in order to solve the problem of translating mouse and monkey transfection and electroporation protocols to human ES cells, but no one was successful prior to the instant invention. See p. 10, #20.

This is not persuasive. Firstly, Zwaka does not state that electroporation did not work, as asserted by the Declaration. Although they teach that transfection rates are low, there is no evidence that there was no transfection of the hES cells. As stated previously, the claims do not require a particular yield or transfection efficiency, thus, arguments directed to these aspects are not found to be pertinent to the rejections of record. Whether or not the levels of transfected cells are practical, are not within the scope of the claims. The claims merely require that some – even one – hES cell is transfected. Thus, the post-cited art of Zwaka, the instant specification and the Declaration provide evidence that mouse ES cell transfection techniques are feasible for transfection of human ES cells.

Specification

The specification is objected to for the following:

The first line of the specification states that the application gains priority from provisional applications 60/253,222 and 60/267,664. It is unclear what "gains" encompasses. It is suggested that if Applicants intent to claim priority to the provisional applications, the specification be amended to reflect this language.

p. 18, line 5 refers to a page number of the specification. Particularly, it states, "An example protocol is provided on page 25." This is objected to because the specification should not refer to specific page numbers.

Election/Restrictions

This application contains claims 18-35 and 37-47, which were withdrawn from consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected inventions, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in Paper No. 13.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

The prior rejection of claim 48, for the limitation of "foreign genetic material" is withdrawn in view of Applicants' amendment to the claim.

Claims 49-56 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 48, as written, is unclear. The claim recites "derivative cells" in line 9 of the claim. It is unclear what the metes and bounds of "derivative cells" encompasses. For example, are Applicants claiming cells that are differentiated from ES cells? The term "derivative cells" encompasses such cells, as well as, for example, fused cells, which would be a derivative of different cells. Appropriate correction is required. Claims 49-56 depend from claim 48.

Claim 49 recites the limitation "the foreign genetic material" in lines 2-3. There is insufficient antecedent basis for this limitation in the claim. Furthermore, it is unclear which genetic material this refers to as recited in claim 48: DNA not normally present, DNA which occurs in ES cells but not expressed, DNA that occurs in the ES and has been modified, or any DNA that can be modified to be expressed by embryonic cells. Claims 50-56 depend from claim 49.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

The prior rejection of claims 36, 48-56 under 35 U.S.C. 102(a) or 35 U.S.C. 102(e) as being anticipated by Smith *et al.* is maintained for reasons of record. The prior rejection of claims 1-4, 6, 9-13 are withdrawn in view of Applicants' Amendmnet to the claims reciting specific transfection reagents (*e.g.*, cationic non-lipid polymer reagent).

The claims are directed to cell populations comprising a substantially pure population of human ES cells containing an expression altering sequence of exogenous DNA (claim 36), and reagent cell populations for supplying material for transplatntation having altered gene expression, consisting essentially of pluripotent human ES cells modified by genetic DNA which is DNA not normally present in ES cells, which occurs in ES cells, but is not expressed in them at levels which are biologically significant, DNA which occurs in ES cells and has been modified so that it is expressed by selected cells derived from transfected human ES cells, or any DNA that can be modified to be expressed in ES cells, derivative clels alone or in any combination thereof (claim 48). Further embodiments recite specific DNA to be expressed (claims 49-56).

Applicants argue that Smith teaches the use of electroporation to transfect murine ES cells, and does not teach a method of altering gene expression in a

population of human ES cells, wherein the transfection preparation further comprises one or more agents selected from the group consisting of a cationic non-lipid polymer agent, a non-liposomal agent, a cationic lipid agent, as recited in the method claim 1. Thus, Applicants argue that Smith cannot anticipate the instant claims. See pp. 12-13 of the Response.

This is not persuasive. The claims, as currently amended, are product-by-process claims. Where, as here, the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes, the PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product. See In re Ludtke, supra. Whether the rejection is based on "inherency" under 35 USC 102, on "prima facie obviousness" under 35 USC 103, jointly or alternatively, the burden of proof is the same, and its fairness is evidenced by the PTO's inability to manufacture products or to obtain and compare prior art products. In re Best, Bolton, and Shaw, 195 USPQ 430, 433 (CCPA 1977) citing In re Brown, 59 CCPA 1036, 459 F.2d 531, 173 USPQ 685 (1972). Further, see MPEP §2113, "Even though product-by process claims are limited by and defined by the process, determination of patentability is based on the product itself. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process." Thus, Smith anticipates the claimed invention because they teach

methods of transfection of any mammalian embryonic stem cell, which include human ES cells. The method of producing these transfected cells does not depend on its method of production. Accordingly, it is maintained that Smith anticipate the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-4, 6, 7, 11-16, 36, 48-56 are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.*, in view of Fasbender *et al.*, cited in the prior Office actions.

The claims are directed to methods of altering gene expression of a population of human ES cells comprising, introducing a transfection preparation comprising a polynucleotide into a population of cells, wherein a) the polynucleotide is operably linked to a promoter and contains a gene expression altering sequence so that gene expression in the ES cells prior to introducing the polynucleotide is measurably different from gene expression after introducing the polynucleotide while retaining the pluripotent character of the cells; and b) the transfection preparation further comprises one or more transfection reagents selected from the group consisting of a cationic non-lipid polymer reagent, a non-liposomal reagent, a cationic lipid reagent.

Smith teaches the generation of genetically modified stem cells. The stem cells include both unipotential and pluripotent stem cells, embryonic stem cells, etc. See col. 2, lines 12-15. Smith teaches that the cells can contain a selectable marker which is capable of differential expression in stem cell and cells other than the desired stem cells, wherein the differential expression of the selectable marker results in preferential isolation and/or survival and/or division of the desired stem cells. They teach that the term "animal cell" embraces all animal cells, including human cells. See col. 2, lines 1-11. In particular, Smith teaches that a positive

selectable marker or a negative selectable marker may be used in transfecting the cells. For example, a foreign gene, a cellular gene, or an antibiotic resistance gene, such as neomycin. See col. 2, lines 25-29. They further teach that various means of introducing the selectable marker may be employed, such as transfection, viral vector, lipofection, or by electroporation. See col. 2, lines 61-64. Smith do not specifically teach the formulation of the polynucleotide with a cationic non-lipid polymer transfection reagent for introduction into the stem cells.

However, prior to the time the claimed invention was made, Fasbender teach methods of transfecting various cell types utilizing complexes of cationic molecules and adenovirus, which was found to enhance gene transfer *in vitro*. See Abstract. Fasbender teach COS-1, NIH-3T3 and 9L gliosarcoma cell cultures were used for the methods of transfection involving recombinant adenovirus vectors and various size poly-L-lysine hydrobromide polymers. The cells were subsequently infected and the uptake of the labeled adenovirus was assessed. See *Materials & Methods*. Fasbender teach that the expression of reporter genes was increased in the cultured cells when they were transfected with the combination of viral vector and cationic molecules.

Applicants argue to establish a *prima facie* case of obviousness, there must be 1) suggestion or motivation 2) a reasonable expectation of success and 3) the prior art (or combination) must teach or suggest all of the claim limitations. Applicants argue that in the instant case, these three conditions are not met.

Firstly, Applicants argue that there is nothing that suggests the modifying of the references to arrive at the claimed subject matter, wherein the transfection reagent used to transfect the cells comprises one or more transfection reagents selected from the group consisting of cationic polymer reagent, as required in claim 11. Applicants argue that in order to arrive at the presently claimed subject matter, there would have to be a suggestion or motivation to modify every known reference or protocol relating to transfection in mammalian cells so that one could achieve successful transfection in human ES cells. See p. 13, 3rd full ¶. Applicants further argue that, "It is just not believable that someone would find the motivation in the knowledge generally available to combine all the necessary references (protocosl) and concomitantly modify each of them to arrive at all of the limitations of the presently claimed invention, since there is no suggestion in any of the cited prior art references to modify them or combine them with another. See p. 14, 1st ¶.

This is not persuasive. In response to Applicant's argument that there is no suggestion to combine the references, the examiner recognizes that obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. See *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988)and *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992). In this case, Smith teaches that means of introducing the DNA in

ES cells can be by methods such as transfection, viral vector, lipofection, or by electroporation. Fasbender teach transfection using a viral vector and a cationic non-lipid polymer. Thus, there is no need to "modify every known reference or protocol relating to transfection" as argued by Applicants, because Smith teaches various methods, such as utilizing a viral vector, which can be used to transfect ES cells, and Fasbender provides methods utilizing a viral vector with poly-L-lysine hydrobromide polymers, showing that using this combination increases the transfection efficiency. See also Fasbender, who state that, "[T]he complexes of adenovirus and cationic molecules increase the efficiency of gene transfer." See Abstract.

Applicants further argue that Fasbender deals with the transfection of genes into COS-1 cells and NIH-3T3 cells or 9L gliosarcoma cells (all of which are different cells), and not with the transfection of human ES cell lines. Applicants argue that none of the cell lines used in Fasbender were stem cells, let alone human ES cells. Applicants argue that one of skill in the art, "is not going to simply mix and match transfection protocols from such disparate references", namely Smith, dealing with electroporation transfection methods in animal ES cells, and Fasbender, dealing with adenovirus-coupled transfection techniques in the presence of cationic molecules in non-human, non-stem cell systems. See p. 14-15 of the Response.

This is not persuasive. Firstly, Smith provides teachings not only for electroporation, but various other methods well-known to those in the art as methods for transfection of cells. These include electroporation, but also transfection, lipofection, injection, ballistic missile and viral vectors. See col. 2, lines 61-64. Thus, they clearly contemplate utilizing various methods to introduce DNA into ES cells. Fasbender provides evidence that utilizing adenoviruses and cationic molecules increases transfection efficiencies in various cell types. Thus, it is maintained that it would have been obvious, given the teachings of Smith, who clearly teach that various methods may be used to introduce DNA into ES cells, to utilize, the specific transfection method, as taught by Fasbender, with a reasonable expectation of success.

Applicants further argue that Fasbender would have to be modified to eliminate the adenovirus from the transfection methodology, but there is no suggestion or motivation to do so. Thus, Applicants argue that the combined references do not teach removing the adenovirus from the transfection protocol are only plausible using impermissible hindsight. See p. 15, 1st ¶ of the Response.

This is not persuasive. There is no specific recitation in the claims, as instantly amended, that would eliminate utilizing an adenovirus. In fact, the breadth of the claims encompasses transfection of the ES cells with or without adenovirus. The claims are silent with regard to the presence or absence of adenoviruses in the transfection preparation. In response to applicant's argument

that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

Applicants argue that there is no reasonable expectation of success with regard to the combination of the references (Smith and Fasbender, and Pascolo for claim 17) because Fasbender deals with the transfection into COS-1 cells and NIH-3T3 cells or 9L gliosarcoma cells, not transfection into human ES cells. Applicants argue that because none of the cells were stem cells, let alone human stem cells, and Smith teaches electroporation. Further, in assuming that one of skill in the art would find motivation to combine the reference and modify both of the references extensively to arrive at a transfection protocol using transfection reagents from cationic molecules, and not using electroporation or adenovirus as a co-reagent for introducing the genetic material, one of skill in the art would have no expectation that the resulting protocol would succeed in transfecting genetic material into hES cells. See pp. 15-16 of the Response.

This is not persuasive. Firstly, the arguments of counsel cannot take the place of evidence in the record. See *In re* Schulze, 346 F.2d 600, 602, 145 USPQ

716, 718 (CCPA 1965) and MPEP §716.01. Thus, merely stating that one of skill in the art would have no success in transfecting the hES cells is not found to be persuasive. It is reiterated that the instant claims are rendered obvious over the cited art of record, because it would be obvious for one of ordinary skill in the art to utilize the methods of transfecting stem cells, as taught by Smith in combination with the teachings of Fasbender, with a reasonable expectation of success. The methods of transfection, as contemplated by Smith and Fasbender, would be methods known to those skilled in the art. The claims do not require a particular number of cells, or a particular transfection efficiency and the claims encompass a single hES cell transfected by the claimed method. Thus, one of ordinary skill would have a reasonable expectation of success given the teachings of both Smith and Fasbender.

Applicants argue that in the highly unpredictable field of stem cell research, one cannot assume that a protocol that works in one animal will work in another and there is no expectation, given the poor transferability of mouse experiments to human experiments, that transfection procedures that are optimized for mice, could be modified to successfully transfect genetic material into human ES cells and result in the transfected genetic material altering gene expression in the hES cells but still retaining the pluripotent character of the hES cells. Applicants argue that as of the filing date, no one had shown that mouse protocols for ES cells, whether

transfecting genetic material, or for performing other manipulations, could be successfully translated to human ES cells. See p. 16 of the Response.

This is not persuasive. It is acknowledged that field of stem cells is unpredictable, for example, the isolation of ES cells from various species is highly unpredictable. However, the introduction of DNA into cells is routine and predictable. Furthermore, as evidenced by Applicants' submission of post-filing art cited in the prior Office action of Zwaka and Thomson provide evidence that, for example, electroporation methods which are successfully used in mouse protocols can be used to produce transfected human ES cells. See prior Office action, p. 13-14 and Applicants' Response filed 12/22/03, p. 14. Thus, it is maintained that the cited art provides sufficient motivation to combine, and a reasonable expectation of success.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Claims 5 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.* when taken with Fasbender as applied to claims 1-4, 6, 7, 11-16, 36, 48-56 above, and further in view of Myers.

The claims are directed to methods of altering gene expression of a population of human ES cells comprising, introducing a transfection preparation

comprising a polynucleotide into a population of cells, wherein a) the polynucleotide is operably linked to a promoter and contains a gene expression altering sequence so that gene expression in the ES cells prior to introducing the polynucleotide is measurably different from gene expression after introducing the polynucleotide while retaining the pluripotent character of the cells; and b) the transfection preparation further comprises one or more transfection reagents selected from the group consisting of a cationic non-lipid polymer reagent, a non-liposomal reagent, a cationic lipid agent. In further embodiments, the protein is a fluorescent protein selected from green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein, and yellow cyan protein.

Smith and Fasbender are described *supra*. They do not teach that the gene product encodes a fluorescent protein such as green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.

However, prior to the time the claimed invention was made, Myers teaches that bioluminescent and chemiluminescent reactions are used as analytical tools in various analytical applications, such as reporter gene studies. See p. 165, 2nd column, 1st ¶. Myers teaches that bioluminescent genes include the firefly luciferin and Renilla [see p. 165, 2nd column, lines 14-17 and #2]. Myers teaches that the gene for firefly luciferase has been cloned and is an effective reporter gene for studying transcriptional activity of cloned genomic sequences. See p. 168, #3.2.

Accordingly, in view of the combined teachings, it would have been obvious for one of skill in the art to utilize the methods of transfecting stem cells, as taught by Smith and Fasbender, and transfect a construct encoding a fluorescent protein, such as Rennila protein, or luciferase, with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as it was well-known in the art to use such fluorescent proteins as reporter genes and various other assays, and as supported by Myers, "Bioluminescent reactions are used as analytical tools in protein and nucleic acid blotting, in nucleic acid sequencing and hybridization assays, and in reporter gene studies ... The main advantages to these reactions are their simplicity and analytical sensitivity." See p. 165, 2nd column, 1st ¶.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Claim 17 is rejected under 35 U.S.C. 103(a) as being unpatentable under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.* in view of Fasbender *et al.* as applied to claims 1-4, 6, 7, 11-16, 36, 48-56 above, and further in view of Pascolo *et al.* is *maintained* for reasons of record.

Applicants argue that the combination of the references, and the required modifications – particularly removing adenovirus from the transfection protocol

directly against the teachings of Fasbender – are only plausible using impermissible hindsight, an advantage not available when combining/citing references for an obviousness rejection. See p. 15 of the Response.

This is not persuasive. Firstly, the claims do not state that the adenovirus is removed from the transfection protocol. The claims recite "a transfection preparation comprising ....". It is noted that the transitional phrase "comprising" is considered open language. See MPEP §2111.03. The arguments with regard to Fasbender have been address in the rejection above.

Applicants argue that Pascolo is directed to the teachings of knockout genomic sequences, but do not provide teachings about the transfection of human ES cells, and thus, when combined with Smith and Fasbender, all the claim limitations of the present invention are not taught or suggested by the combinations. See p. 17, 3rd full ¶ of the Response.

This is not persuasive. As stated in the prior Office actions and preceding paragraphs, Smith and Fasbender provides teachings with regard to transfection of human ES cells that fulfill the limitations of the claims with regard to human ES cells. Furthermore, Pascolo provides the motivation to knock-out endogenous genes to analyze gene expression and, and that in generating the double knockout H-2D^b /mouse beta2 microglobulin, Pascolo states, "This should facilitate the study of HLA class I-restricted responses compared to classical transgenic mice. One might home

that the information gained with these animals will be of human relevance." See p. 2050, 2nd column, lines 4-7.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Claims 8 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.* in view of Fasbender *et al.* as applied to claims 1-4, 6, 7, 11-16, 36, 48-56 above, and further in view of the Gibco BRL catalog.

Applicants argue that the combination of art do not teach or suggest all of the claim limitations of the presently claimed subject matter as currently amended.

This is not found to be persuasive. It is maintained that Smith provides sufficient teachings with regard to transfection of ES cells, and specifically hES cells. Smith and Fasbender are described *supra.* The Gibco catalog teaches LIPOFECTIN®, which is a liposomal formulation of a cationic lipid which is used to transfect a wide variety of cells, including human cells. See 1st ¶.

Accordingly, it is maintained that in view of the combined teachings of Smith, Fasbender and the Gibco BRL catalog, it would have been obvious for one of skill in the art to utilize the methods of transfecting human ES cells, as taught by Smith, by using a transfection reagent, such as LIPOFECTIN®, with a reasonable expectation of success. One of ordinary skill in the art would have been motivated

to make such a modification, as it was an art-recognized goal to optimize transfection techniques of mammalian cells, and, as supported by the Gibco BRL catalog, that the LIPOFECTIN® reagent is a more efficient method of transfecting cells than calcium phosphate or DEAE-dextran transfection methods.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

The prior rejection of claims 1-4, 6, 10, 36, 48, 52, 54 and 56 under 35 U.S.C. 103(a) as being unpatentable over Thomson when taken with Bradley *et al.* is withdrawn in view of Applicants' amendment to the claims with recitation of specific transfection reagents (*i.e.,* cationic non-lipid polymers, non-liposomal reagents, cationic lipid agent).

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Thaian N. Ton whose telephone number is (571) 272-0736. The Examiner can normally be reached on Monday through Friday from 8:00 to 5:00 (Eastern Standard Time), with alternating Fridays off. Should the Examiner be unavailable, inquiries should be directed to Amy Nelson, Acting SPE of Art Unit 1632, at (571) 272-0804. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703) 872-9306.

tnt
Thaian N. Ton
Patent Examiner
Group 1632

Joe Woitach
AU1637